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Short Communication

Determination of vitamin D_2 in shiitake mushroom (*Lentinus edodes*) by high-performance liquid chromatography

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ABSTRACT

The total content of vitamin D_2 (ergocalciferol) in shiitake mushroom (*Lentinus Edodes*) was determined by high-performance liquid chromatography. The vitamin D_2 content fluctuated considerably in different years of harvest and according to the brands and the quality of grades; the reason may be that most shiitake mushroom are cultivated under natural climatic conditions.

INTRODUCTION

Ergocalciferol (vitamin D_2; D_2) is contained in shiitake (*Lentinus edodes*), a kind of edible mushroom cultivated widely in Japan [1], and its determination is therefore important from the nutritional point of view.

D_2 has been determined in shiitake by spectrophotometric [2,3] gas–liquid chromatographic [4] and high-performance liquid chromatographic (HPLC) procedures [5,6]. However, significant differences in D_2 contents in shiitake were reported, because a small number of samples were taken for determination, the determinations were not conducted on samples taken in consecutive years of harvest but at random, different kinds of pretreatment of the samples were applied or very sensitive detectors (absorbance $1 \cdot 10^{-3}$), generally not in common (sensitivity $1 \cdot 10^{-2}$) use, were used[a].

In this work, the above differences in D_2 contents in shiitake obtained using HPLC procedures were investigated. The results obtained for the determination of D_2 in shiitake by HPLC procedure are discussed with regard to the quality of grades, the consecutive years of harvest and the various brands.

[a] The mobile phase of D_2 determination by HPLC used mainly a solution of *n*-hexane. It is difficult to detect high sensitivity for an unstable baseline.

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EXPERIMENTAL

The test samples used were obtained from cultivated Japanese shiitake mushroom, harvested and heated to dryness in the years 1986–88. The D_2 contents in the samples were determined by applying the HPLC procedure in the year of harvest. A 10-g sample was homogenized in a blender and an aliquot of *ca.* 3 g of the homogenate was placed in a digestion flask. After the addition of 40 ml of aldehyde-free ethanol, 4 g of pyrogallol and 10 ml of 50% potassium hydroxide solution the sample was decomposed at 80°C for 30 min. After the mixture had cooled completely it was extracted with 100 ml of benzene. The benzene fraction was washed once each with 100 ml of 1 and 0.5 *M* potassium hydroxide solution and four times with 30 ml of distilled water and then filtered through Whatman 1PS filter-paper. An aliquot of 90 ml of the filtrate was evaporated to dryness below 35°C. The residue was dissolved in 1 ml of methanol–acetonitrile (1:1) and the solution obtained served as the test material for the determination of D_2 by HPLC.

A 200-μl volume of the solution was injected onto a LiChrosorb RP-18 column (250 mm × 7.5 mm I.D.) and eluted with methanol–acetonitrile (1:1) at a flow-rate of 2.2 ml/min. The fraction obtained was evaporated to dryness and the residue was dissolved in 0.5 ml of the HPLC eluent. For the determination of D_2 by HPLC an aliquot (30 μl) of the solution was injected onto to a Nucleosil 100-5 column (150 mm × 4.6 mm I.D.) and eluted with hexane containing 0.1% of *n*-amyl alcohol and 0.4% of isopropyl alcohol at a flow-rate of 1 ml/min. An NSLC Model 100A HPLC unit (Nippon Seimitsu Kagaku) was used; reagents used were obtained from Wako Junyaku Kogyo.

RESULTS AND DISCUSSION

A chromatogram obtained from the test material by preparative HPLC is shown in Fig. 1. A fraction containing D_2 was obtained with a retention time of 18–22 min, and before and after this fraction other benzene-soluble substances were eluted. As shown in Fig. 2, when the fraction containing D_2 (*ca.* 50 ng) was subjected to HPLC, a single peak of D_2 with a retention time of 7.6 min was obtained. From the results obtained, it is evident that D_2 is separated satisfactorily on the 150-mm column and the sensitivity of the absorbance detector was 1×10^{-2}. The results of a recovery test with authentic D_2 are shown in Table I. Takeuchi *et al.* [7] reported that D_2 detected in dried siitake was mostly in the free form and the esterified form was not detected. From these results, it may be better to apply a direct saponification method than an extraction method [6] for crude fat in test material.

Determination of total vitamin D_2 content in dried shiitake

The results of the determination of total D_2 content (D_2 + pre-ergocalciferol) in dried shiitake obtained in consecutive years and according to the brand and the quality of the grades are shown in Table II. Kiribuchi [6] reported that D_2 was contained in some samples of dried siitake but not in others. In this work, however, the existence of D_2 in all the samples tested was confirmed but the yearly fluctuations of the total content of D_2 were significant.

According to the report of Takeuchi *et al.* [7], the total content of D_2 was higher

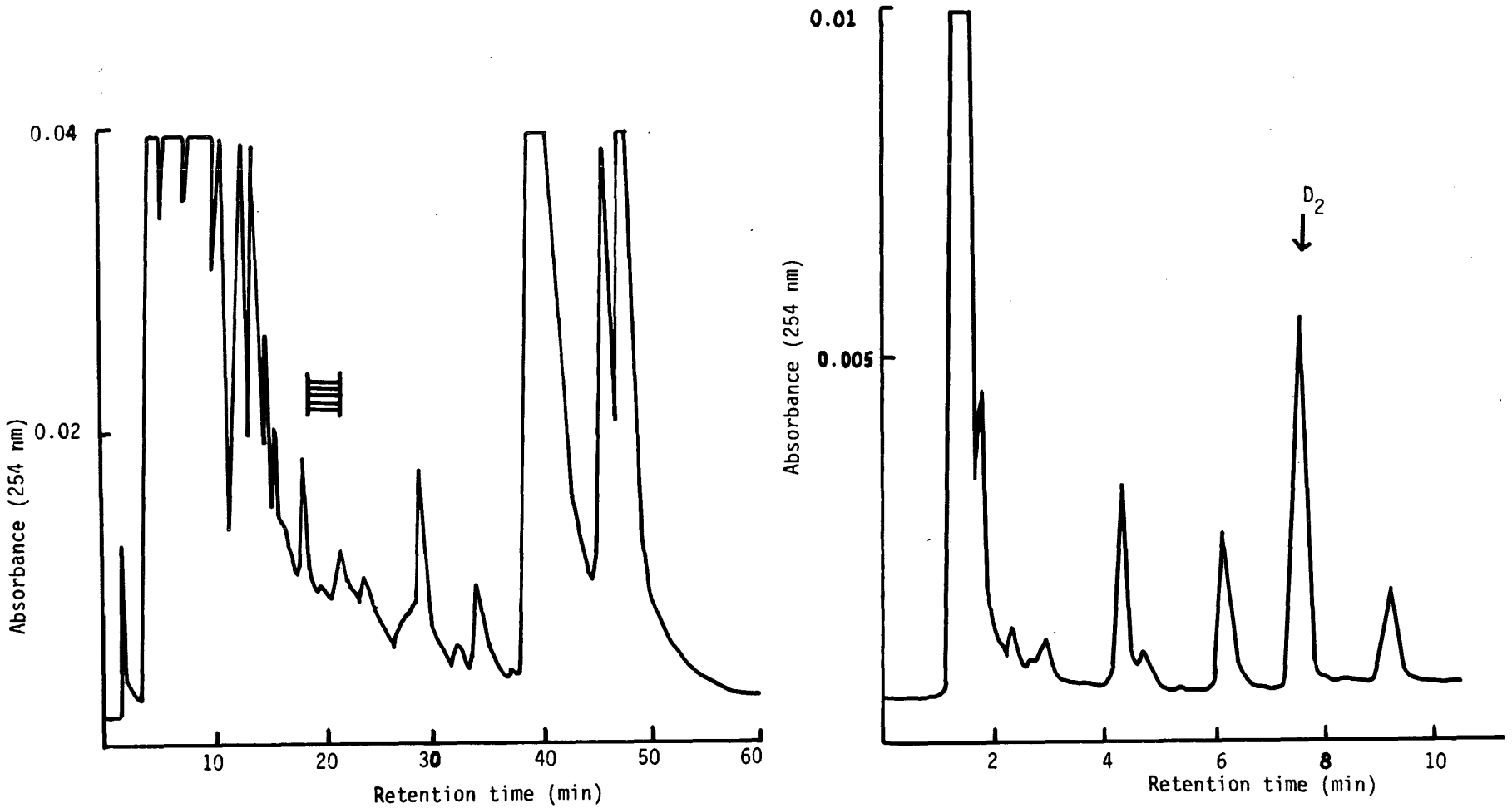

Fig. 1. HPLC of D_2 in crude extract of dried shiitake, obtained with clean-up procedure. LiChrosorb RP-18 column, 250 mm × 7.5 mm I.D.; mobile phase, methanol–acetonitrile (1:1); flow-rate, 2.2 ml/min. Hatched area: the fraction with the same retention time as that of the standard sample was collected.

Fig. 2. HPLC of purified D_2 fraction obtained from dried shiitake. Nucleosil 100-5 column, 150 mm × 4.6 mm I.D.; mobile phase, *n*-hexane containing 0.1% *n*-amyl alcohol and 0.4% isopropyl alcohol; flow-rate, 1 ml/min; UV detection at 254 nm.

TABLE I

DETERMINATION AND RECOVERY TEST OF VITAMIN D_2 IN DRIED SHIITAKE BY HPLC

Trial No.	Vitamin D_2 (IU per 100 g)		Recovery (%)
	Added	Total present[a]	
1	1500	3206	94.8
2	1500	2906	74.8
3	1500	2960	78.4
4	1500	3118	88.9
5	1500	3014	82.0
Mean ± S.D.			83.8 ± 7.2

[a] Without addition of vitamin D_2, mean ± S.D. ($n=5$) = 1784.4 ± 108.1 IU per 100 g.

TABLE II

CONTENTS OF VITAMIN D_2 IN DRIED SHIITAKE BY HPLC

Brand name[a]	Vitamin D_2 (IU per 100 g dry wt.)[b]		
	1986	1987	1988
Jyo-donko	1516 ± 932	1752 ± 568	1090 ± 154
Nami-donko	2767 ± 285	873 ± 68	1397 ± 346
Kotsubu-donko	3102 ± 928	1457 ± 260	1182 ± 396
Jyo-koshin	2026 ± 792	4382 ± 379	1809 ± 158
Nami-koshin	2062 ± 570	1762 ± 411	1607 ± 99
Chayori	1828 ± 83	1871 ± 437	2148 ± 560

[a] Jyo, high grade; Nami, middle grade; Kotsubu and Chayori, low grade.
[b] Values are means ± S.D. for 15 samples.

in Koshin (mushroom with large pileus) than in Donko (mushroom with small pileus) and this tendency was observed in general, but no significant differences were observed. The reason why the total contents of D_2 in the dried shiitake fluctuate significantly in different years of cultivation and according to the brands and quality of grades may be that most shiitake mushrooms are cultivated under natural climatic conditions.

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